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A Single-Amino-Acid Change of the Gustatory Receptor Gene, *Gr5a*, Has a Major Effect on Trehalose Sensitivity in a Natural Population of *Drosophila melanogaster*

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ABSTRACT

Variation in trehalose sensitivity and nucleotide sequence polymorphism of the *Gr5a* gene encoding the gustatory receptor to sugar trehalose were investigated in 152 male lines of *Drosophila melanogaster* collected from a natural population. Among the observed 59 segregating sites, some pairs of sites showed significant linkage disequilibrium. A single SNP, which results in the Ala218Thr amino acid change, was significantly associated with trehalose sensitivity, as previously suggested. Threonine at amino acid position 218 was found to be the ancestral form in *D. melanogaster*, suggesting that low trehalose sensitivity was an ancestral form with respect to the receptor function. There was large genetic variation in trehalose sensitivity. It was continuously distributed, indicating that trehalose sensitivity measured by the behavioral assay is a quantitative trait. These results suggest that apart from the *Gr5a* gene, other genetic factors contribute to variation in trehalose sensitivity. Nucleotide diversity (π) and nucleotide variation (θ) per site were 0.00874 and 0.00590, respectively. Fu and Li's test and the MK test showed no significant departure from the expectation of selective neutrality in the *Gr5a* gene. However, we rejected selective neutrality by Tajima's test and Fay and Wu's test with the observed level of recombination. We discuss possible causes of the observed pattern of nucleotide variation in the gustatory receptor *Gr5a* gene.

GUSTATORY information is considered to be important for animals to control behavior when they search for food or partners. Recently, candidates of gustatory receptors in Drosophila have been identified on the basis of their structure and specific expression patterns (Clyne *et al.* 2000; Dunipace *et al.* 2001; Scott *et al.* 2001). They consist of a large multigene family encoding putative G protein-coupled receptors, which have seven-transmembrane domains (Clyne *et al.* 2000). Thus far, 60 members of gustatory receptor (*Gr*) genes, which encode 68 Gr proteins through alternative splicing, have been reported (Robertson *et al.* 2003). However, little is known about the specific taste ligand molecules that interact with or activate each gustatory receptor.

A gustatory receptor encoded by the *Gr5a* gene has been confirmed to be necessary for reception of a disaccharide, trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) in *Drosophila melanogaster* (Dahanukar *et al.* 2001; Ueno *et al.* 2001; Chyb *et al.* 2003). It has been molecularly and physiologically characterized (Dahanukar *et al.* 2001; Ueno *et al.* 2001). The product of the *Gr5a* gene was expressed in taste neurons of the labelum and tarsi, and it showed ligand specificity for trehalose (Chyb *et al.* 2003). Thus, it has been the first invertebrate taste receptor functionally characterized (Dahanukar *et al.* 2001; Ueno *et al.* 2001; Chyb *et al.* 2003). A locus, *Tre* (*Trehalose sensitivity*), was found to be a genetic dimorphism (high or low sensitivity) among laboratory strains, and the *Tre* locus was mapped on the *X* chromosome (Tanimura *et al.* 1982). The *Tre* locus controls electrophysiological sensitivity of the labelar gustatory neurons as well as the feeding preference to trehalose. The sugar is present in yeasts and fungi, which are important food sources for Drosophila, and it is also used as blood sugar in insects. Interestingly, the *Tre* locus controls taste sensitivity specifically to trehalose but not to other sugars like sucrose or fructose (Tanimura *et al.* 1982). At the *Tre* locus the *Gr5a* gene was identified, which encodes the gustatory receptor of trehalose (Dahanukar *et al.* 2001; Ueno *et al.* 2001). An amino acid change (Ala218Thr) in the *Gr5a* gene appears to be associated with dimorphic phenotypes, Tre^{+} (high sensitivity) and Tre^{01} (low sensitivity), in trehalose sensitivity (Ueno *et al.* 2001). However, we do not know the patterns of genetic variation in trehalose sensitivity

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and nucleotide sequence variation of the *Gr5a* gene in natural populations. We also do not know which part of naturally occurring variation in the *Gr5a* gene is associated with trehalose sensitivity and what the selective significance of this variation is. To answer the above questions, we examined the pattern of trehalose sensitivity and nucleotide sequence variation of the *Gr5a* gene in 152 male lines collected from a natural population of *D. melanogaster*.

Here we show that there was genetic variation in trehalose sensitivity, and that it was continuously distributed. We found that a single single-nucleotide polymorphism (SNP) in the *Gr5a* gene, which results in the amino acid change, was significantly associated with trehalose sensitivity, as previously suggested (Ueno *et al.* 2001). Furthermore, we found that apart from the *Gr5a* gene, other genetic factors contribute to variation in trehalose sensitivity. Selective neutrality was rejected by Tajima's test and Fay and Wu's test with the observed level of recombination. We discuss the possible causes of the observed pattern of nucleotide variation in the gustatory receptor *Gr5a* gene.

MATERIALS AND METHODS

Fly samples: Male flies of *D. melanogaster* were collected from a natural population in Kyoto, Japan, in July 2002. Each male fly was crossed to females of the attached-*X* chromosome strain, *C(1)DX, ywf*. Therefore, the *X* chromosome of a parental male fly is inherited to male offspring. After crossing, parental males were collected and used to extract genomic DNAs. Parental females were transferred to a new vial every 3 days to allow them to lay eggs. Three independent vials were kept per line and used for measurement of trehalose sensitivity. Males from the F_1 generation were used to measure trehalose sensitivity. In this study, 152 male lines were used for measurement of trehalose sensitivity and nucleotide sequence analyses. Twelve Zimbabwean strains of *D. simulans* were provided by the National Institute of Genetics, Japan.

DNA extraction, PCR, and sequencing: Genomic DNA was extracted from single male flies using the GenElute mammalian genomic DNA kit (Sigma, St. Louis). About 1.8 kb of the *Gr5a* gene region on the *X* chromosome of *D. melanogaster*, which consists of a 5′-flanking region, seven exons, and six introns, was amplified by the PCR method with primers TreFor, 5′-CTGTTTTATTCCTCATCACTGGCC-3′ and TreRev, 5′-TACATGCCAATTAGTGCGTCT-3′. Because the entire *Gr5a* gene region of *D. simulans* could not be amplified using these primers, an ~1.3-kb-long partial region including the 5′-flanking region was amplified by the following primers: TreFor, 5′-CTGTTTTATTCCTCATCACTGGCC-3′ and TreR2, 5′-ATGAAGTACAGATTGCTGCC-3′. The PCR reaction conditions for 32 cycles were denaturing at 95° for 30 sec, annealing at 60° for 30 sec, and polymerizing at 72° for 1 min. To minimize the effect of PCR errors, two 50-μl PCR reactions for each line were pooled and purified using the Wizard SV gel and PCR clean-up system (Promega, Madison, WI). Direct sequencing was performed using an ABI (Columbia, MD) model 3100 automated sequencer and DNA sequencing kit (BigDye terminator cycle sequencing ready reaction version 3). Both strands of PCR products were sequenced using the following eight synthetic oligonucleotide primers: TreFor, 5′-CTGTTTTATTCCTCATCACTGGCC-3′; TreF2, 5′-TGGGTGAGATTAAATGATGC-3′; TreF3, 5′-TGCTGCTCACCTTCGGCTGG-3′; TreF4, 5′-ACTTCTCGCTGCTGTTCCTG-3′; TreRev, 5′-TACATGCCAATTAGTGCGTCT-3′; TreR2, 5′-ATGAAGTACAGATTGCTGCC-3′; TreR3, 5′-ATGCTCAGCAGGTGTTCCAC-3′; and TreR4, 5′-TGTACCAGAATCGCCAGCTG-3′. All sequences obtained in this study were deposited in the DDBJ with accession nos. AB162449–AB162612.

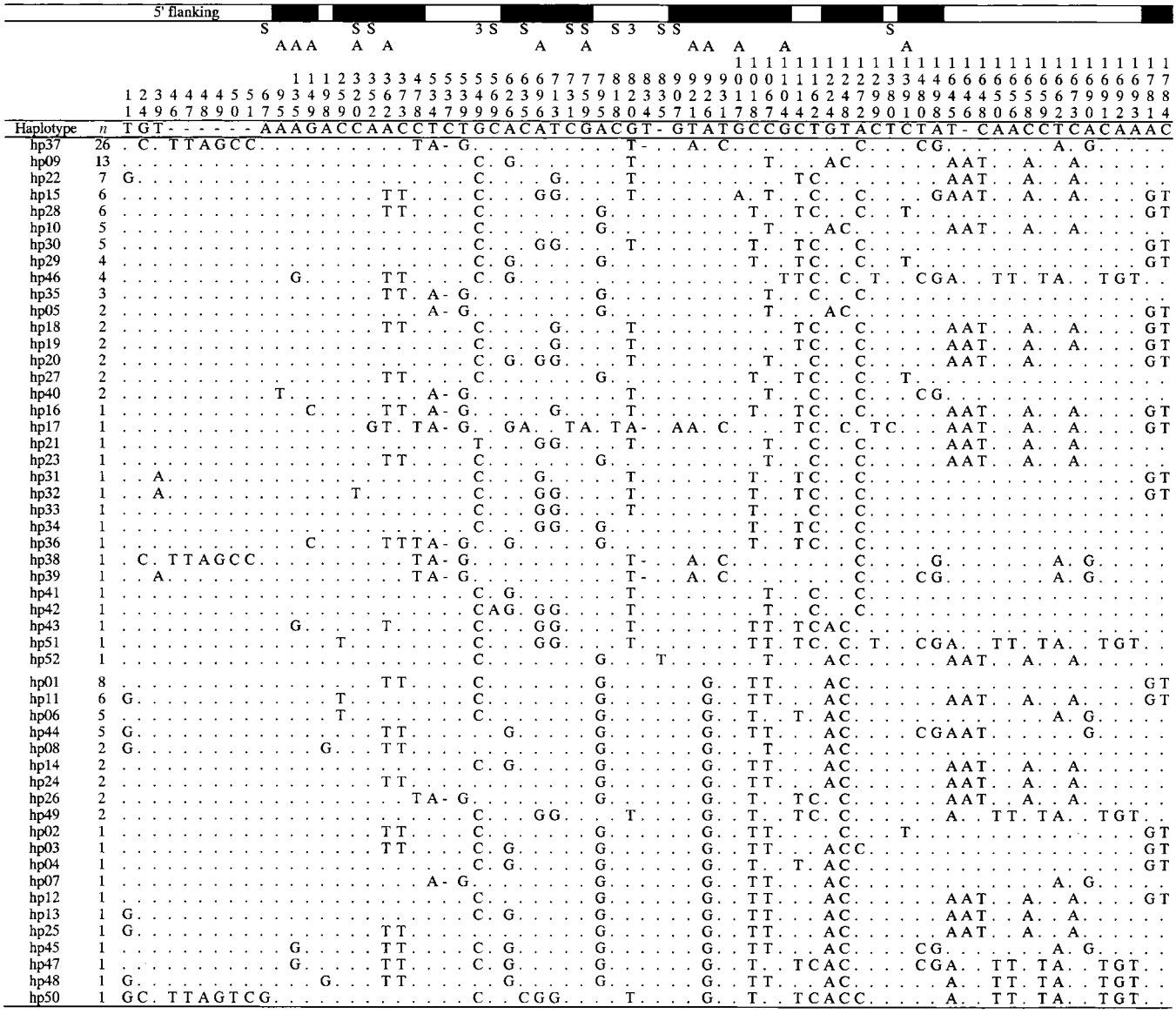
Measurement of trehalose sensitivity: Trehalose sensitivity was measured by the two-choice preference test described in detail by Tanimura *et al.* (1982). The test measures relative sensitivity to trehalose with respect to the sweetness of sucrose. Food-deprived flies show vigorous feeding and can be easily visualized by mixing food dye with sugar solutions. Flies were allowed to choose 20 mm trehalose solution with a blue food dye (0.125 mg/ml brilliant blue FCF) or 2 mm sucrose solution with a red food dye (0.5 mg/ml acid red 27) in a microtest plate. Flies fed 20 mm trehalose, 2 mm sucrose, or both solutions showed blue, red, or purple abdomens, respectively. The test was done in the dark to avoid visual discrimination. The reciprocal combinations of the sugar and food dyes give similar results, indicating that the preference is based predominantly on sugar solutions rather than on the food dye mixed with the solutions. After 1 hr feeding, only male flies with different abdominal colors were counted under the microscope. Trehalose sensitivity was denoted by the preference index (PI), which was defined as the ratio of the sum of the number of male flies with blue abdomen and half the number of male flies with purple abdomen to the sum of the number of male flies with blue, red, and purple abdomens. In this study, flies from the F_1 generation were tested. Three independent tests were carried out for each line.

Data analyses: Nucleotide sequences were edited using the SeqPup program version 0.6f (Gilbert 1996). Sequence alignment was performed using the CLUSTAL X program (Thompson *et al.* 1997). The level of nucleotide variation, linkage disequilibrium (LD), and recombination parameter were measured using the DnaSP program version 4.0 (Rozas *et al.* 2003). Tajima's test (Tajima 1989), Fu and Li's test (Fu and Li 1993), Fay and Wu's test (Fay and Wu 2000), and the McDonald-Kreitman (MK) test (McDonald and Kreitman 1991) were performed using the DnaSP program. We performed 10,000 coalescent simulations with the observed level of recombination [$R = 35.7$ estimated by Hudson's (1987) method], which are implemented in the DnaSP program, to determine the critical values of the test statistics, Tajima's D, Fu and Li's D^*, and Fay and Wu's H.

Analysis of variance (ANOVA) was performed using the StatView software version 4.5. Trehalose sensitivity data were analyzed by one-way ANOVA. The model of ANOVA for the line effect was $Y_i = u + L_i + e_i$, where Y was trehalose sensitivity represented by the PI, u was the overall mean, L_i was the ith male line effect ($i = 1, \ldots, 152$), and e_i was the error term. The line effect was considered as the random effect. Association between nucleotide polymorphism and trehalose sensitivity was assessed by the one-way ANOVA of line means. The model of ANOVA for the allele effect at the segregating site was $Y_i = u + A_i + e_i$, where Y was the line mean of trehalose sensitivity represented by the PI, u was the overall mean, A_i was the ith allele effect at the segregating site ($i = 1$ or 2; G, A, T, or C, $1 =/= 2$), and e_i was the error term. The allele effect at the segregating site was considered as the fixed effect. In the association study, informative sites with alignment gaps were also considered. To assess the interaction effect between the two segregating sites (epistasis) on trehalose sensitivity, two-way ANOVAs of line means were performed. The model of two-way ANOVA was $Y_{ij} = u + A_i + A'_j + (A_i^* A'_j) + e_{ij}$, where Y was the line mean of trehalose sensitivity represented by the

TABLE 1

Segregating sites of the *Gr5a* gene in *D. melanogaster*

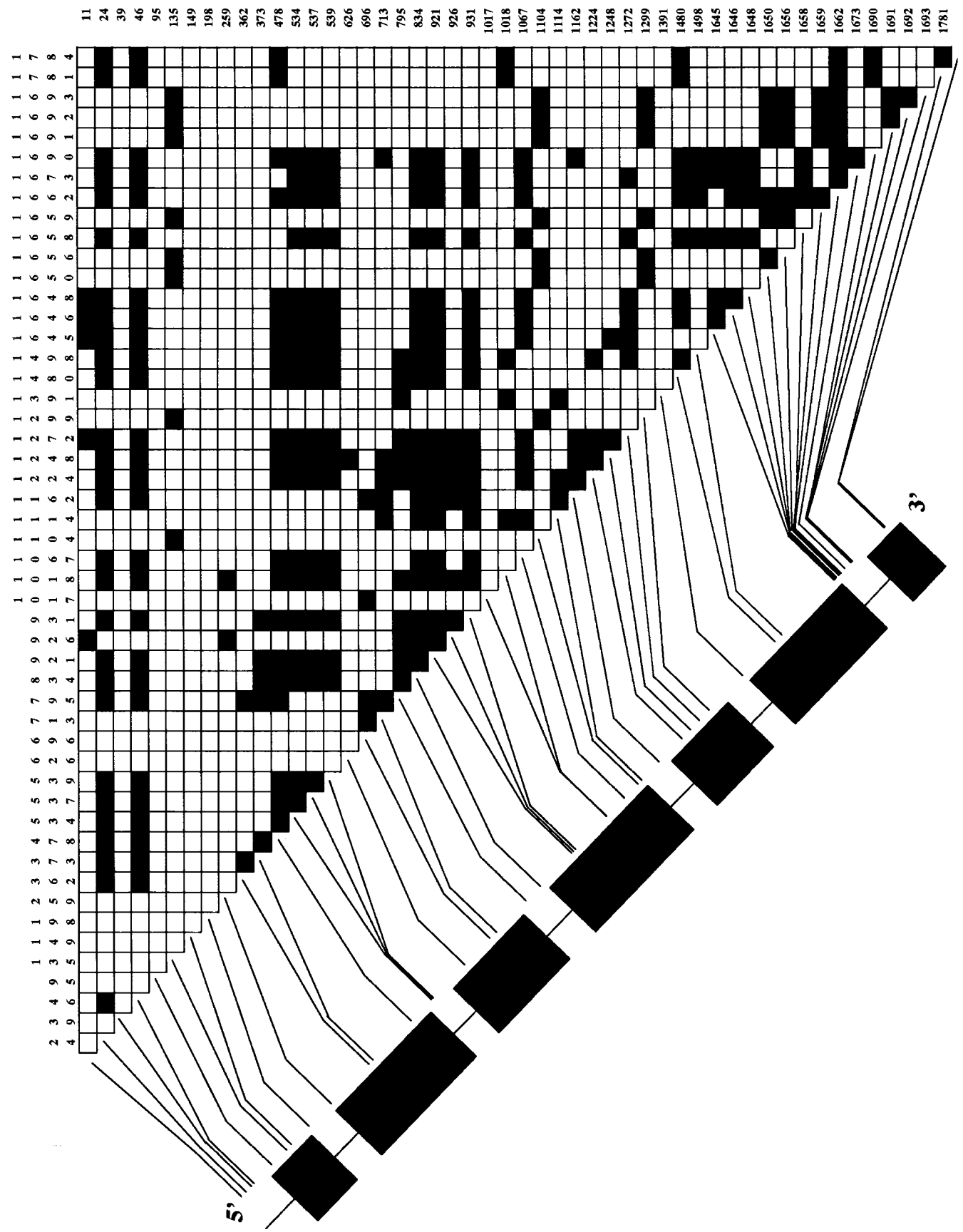


A dot indicates the same nucleotide as a consensus one and a dash indicates deletion. "A," "S," and "3" above the position indicate replacement substitution, singleton, and three variants, respectively. Solid boxes indicate exons. The first position of the start codon (Met) was numbered as +80. *n*, the number of each haplotype in the 152 sequences.

PI, u was the overall mean, A_i and A'_j were, respectively, the ith and jth allele effects at the segregating site (i = 1 or 2; j = 1 or 2; G, A, T, or C, 1 =/= 2), ($A_i^* A'_j$) was the interaction effect between the two segregating sites, and e_{ij} was the error term. All effects were considered as the fixed effect.

RESULTS

Level and pattern of nucleotide variation in the *Gr5a* gene: We determined 152 sequences of the gustatory receptor gene, *Gr5a* in *D. melanogaster*. Table 1 shows segregating sites of the *Gr5a* gene in *D. melanogaster*. The first position of the start codon (Met) was numbered as +80. The sequenced region was 1786 bp long excluding sites with alignment gaps. There were 10 sites with alignment gaps, resulting in four insertions/deletions (indels). One of them was a singleton. All indels were found in the 5′-flanking region and introns. The number of segregating sites was 59. There were 10 singletons and two sites with three variants. Nucleotide diversity (π, Nei 1987) and nucleotide variation (θ, Watterson 1975) per site were 0.00874 and 0.00590, respectively. The numbers of synonymous and replacement changes were 20 and 12, respectively. Nucleotide diversity per silent site (π silent) was 0.01746. There were 52 haplotypes with


5'
3'

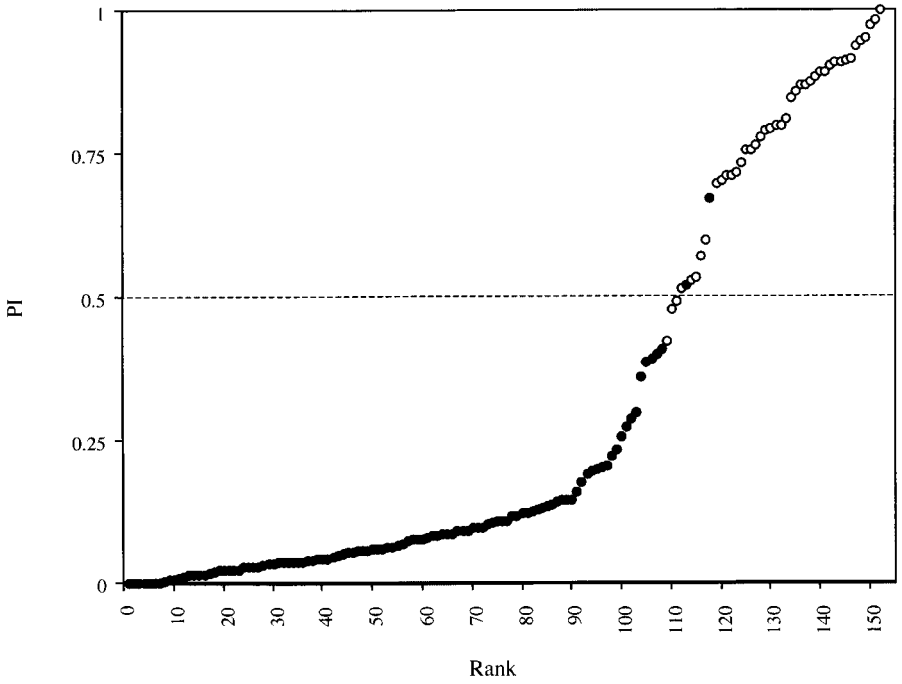


FIGURE 2.—The average PI (trehalose sensitivity) for 152 male lines ranked in increasing order. Solid and open circles indicate the trehalose sensitivity of lines with adenine and guanine, respectively, at position 926.

indels (see Table 1). The frequency of a major haplotype in the 152 sequences was 17.1%. Twenty-seven of the 52 haplotypes appeared only once in the sample, indicating that most haplotypes were rare.

We also determined 12 partial sequences including the 5′-flanking region of the *Gr5a* gene in *D. simulans*, which is the most closely related species to *D. melanogaster*. They were 1239 bp long, excluding sites with alignment gaps. Nucleotide diversity (π) and nucleotide variation (θ) per site were 0.02562 and 0.02833, respectively. Nucleotide diversity per silent site (π silent) was 0.05036. These values were three to five times larger than those of *D. melanogaster*.

The minimum number of recombination events estimated by HUDSON and KAPLAN's (1985) method was 18 for 152 sequences of the *Gr5a* gene in *D. melanogaster*, suggesting that the recombination rate in the *Gr5a* gene region is not low. However, we observed considerable LD among 51 informative segregating sites in 152 sequences of the *Gr5a* gene of *D. melanogaster* even after the conservative Bonferroni correction (Figure 1). We found that site 24 and sites 46–51 (5-bp indel) showed complete linkage. Similarly, sites 534, 537 (1-bp indel), and 539; sites 834 (1-bp indel), 921, and 931; sites 1646 (1-bp indel) and 1648; sites 1650, 1656, and 1659; sites 1691, 1692, and 1693; and sites 1781 and 1784 showed complete linkage.

Variation in trehalose sensitivity: Trehalose sensitivity was measured by the two-choice preference test using 152 male lines from a natural population. Each male line has a single *X* chromosome. We found that trehalose sensitivity was continuously distributed (Figure 2). The PI-values varied from 0 to 1.0. Genetic variation in trehalose sensitivity was highly significant among the lines ($F_{151,304} = 13.145$, $P < 0.0001$). These results indicate that trehalose sensitivity measured by the behavioral assay is a quantitative trait and suggest that there is considerable genetic variation in the taste preference in a natural population.

Association between trehalose sensitivity and nucleotide polymorphism in the *Gr5a* gene: At first assuming random association between segregating sites, we examined association between trehalose sensitivity and nucleotide polymorphisms in the *Gr5a* gene, although as described above there was considerable LD. Because there were seven complete linkages between two or three segregating sites, we performed 40 independent association tests using informative segregating sites. We found that 12 polymorphisms were significantly associated with trehalose sensitivity, eight sites at the 1% level and four sites at the 5% level after the Bonferroni correction (Figure 3A). Two of the 12 polymorphisms were replacement changes. The replacement segregating change (T/A) at position 921, which corresponds to a Leu216His amino acid change, was associated with trehalose sensitivity at the 5% level. This replacement segregating site, however, was completely linked with sites 834 (1-bp indel) and 931. Therefore, not only this replacement change alone may be associated with trehalose sensitivity. The replacement segregating change (G/A) at position 926,

FIGURE 1.—Linkage disequilibrium between segregating nucleotide sites in the *Gr5a* gene. The intron and exon structures are indicated by lines and solid bars, respectively. Only the significance at the 5% level after the Bonferroni correction is indicated by solid boxes.

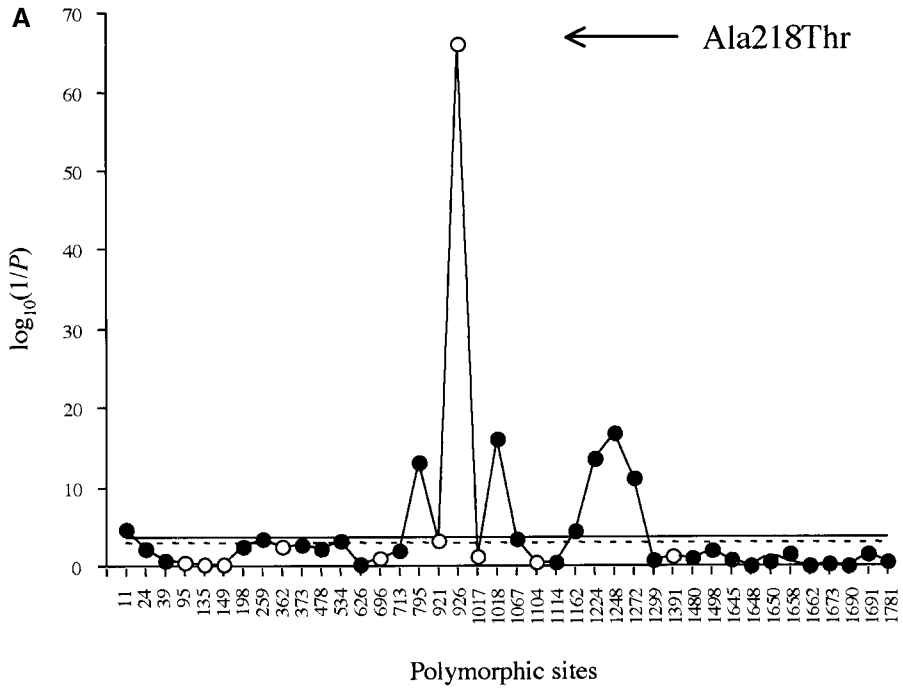


FIGURE 3.—Association of trehalose sensitivity with segregating changes in the *Gr5a* gene for 152 lines (A), 107 lines with adenine at position 926 (B), and 45 lines with guanine at position 926 (C). *x*- and *y*-axes indicate segregating sites and *P*-values of ANOVAs of association, respectively. *P*-values are transformed to $\log(1/P)$. Open circles indicate replacement sites. The dotted and solid horizontal lines indicate the significance at the 5% and 1% levels, respectively, after the Bonferroni correction for multiple tests. Arrow indicates the Ala218Thr amino acid change.

which corresponds to an Ala218Thr amino acid change, showed highly significant association and exceptionally high *P*-value (see Figure 3A).

The 12 polymorphisms observed were in LD. To exclude the effect on association caused by LD between site 926 and the other 11 segregating sites, we performed additional association tests. We tested association between trehalose sensitivity and nucleotide polymorphisms using 107 sequences with adenine at position 926. Similarly, we tested association using 45 sequences with guanine at this position. In both tests, we did not find any association between trehalose sensitivity and nucleotide polymorphism (Figure 3, B and C). Association between trehalose sensitivity and haplotypes harboring the 12 significant polymorphisms was also examined. Significant difference in mean trehalose sensitivity after the Bonferroni correction was found only in comparisons involving haplotypes with the adenine and guanine at position 926, respectively (data not shown). To examine whether there is an interaction effect between segregating site 926 and other segregating sites (epistasis), two-way ANOVA was performed. Out of the 19 tests we found that the interaction effects between sites 696 and 926 and between sites 926 and 1690 were significant at the 5% level ($F_{1,148} = 4.317$, $P = 0.0395$ and $F_{1,148} = 6.535$, $P = 0.0116$, respectively). Nucleotide changes at positions 696 and 1690 were replacement and silent in the sixth intron, respectively. However, we did not find any significant interaction effect after the Bonferroni correction. On the basis of these results, it is difficult to decide whether there is epistasis between amino acid change and silent change in the gustatory receptor function. The observed significant interaction effect might simply have occurred by chance. Figure 2 shows distribution of trehalose sensitivity classified by the replacement segregating change at position 926. The boundary between low and high trehalose sensitivity in terms of the single SNP was ~0.5. This observation was consistent with the genetic dimorphism at the *Tre* locus (TANIMURA *et al.* 1982). These results indicate that a single SNP, which results in the Ala218Thr amino acid change in the *Gr5a* gene, has a major effect on trehalose sensitivity.

We also found that there was considerable genetic variation in trehalose sensitivity among 107 male lines with adenine at position 926 ($F_{106,214} = 1.993$, $P < 0.0001$) and among 45 male lines with guanine at position 926 ($F_{44,90} = 1.831$, $P = 0.0080$), suggesting that apart from the *Gr5a* gene, other genetic factors are responsible for trehalose sensitivity measured by the two-choice preference test.

Nucleotide diversity (π, NEI 1987) per site in the *Gr5a* gene of the 107 male lines with adenine at position 926 and 45 male lines with guanine at position 926 was 0.00824 and 0.00604, respectively. At face value nucleotide diversity of the *Gr5a* gene in the first set of lines was higher than that in the second set. To determine an ancestral form of the single SNP, 12 partial sequences of the *Gr5a* gene of *D. simulans* were obtained. We found that the nucleotide at position 926 in the *Gr5a* gene of *D. simulans* was adenine. Therefore, the adenine at position 926 (threonine at the 218-amino-acid position) was the ancestral form in *D. melanogaster*, suggesting that low trehalose sensitivity was an ancestral form of the receptor function.

Tests of selective neutrality: Frequency spectrum was likely to show an excess of intermediate alleles com-

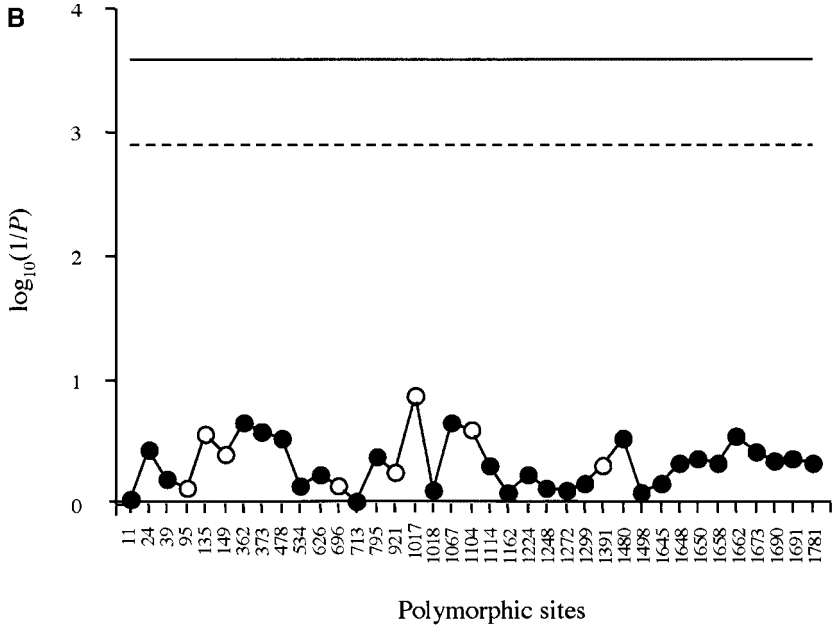
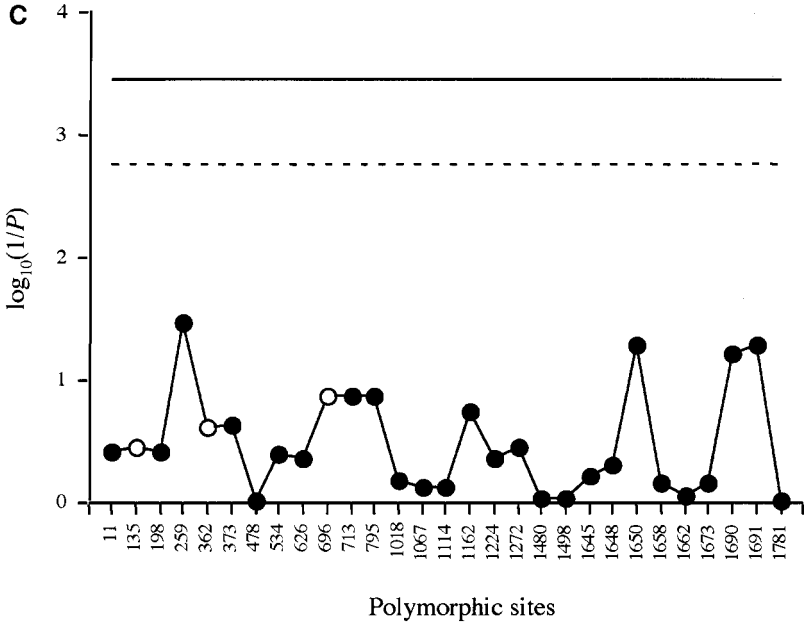


FIGURE 3.—*Continued.*



pared with the neutral expectation (Figure 4). However, Tajima's *D*- and Fu and Li's *D**-values were not significant ($D = 1.3365$ and $D^* = -0.2230$, respectively) under the usual equilibrium neutral model of no recombination. Fay and Wu's test, which detects an excess of high-frequency variants, was carried out using five of the seven exons of *D. melanogaster* and *D. simulans*. The result was not significant ($H = -9.4800$, $P = 0.0517$). The power of these tests is very low in the presence of recombination (*e.g.*, WALL 1999). Because considerable recombination was observed in our sample, 10,000 coalescent simulations with the observed level of recombination [$R = 35.7$ estimated by HUDSON's (1987) method] were performed to determine the critical values of the test statistics. We found that Tajima's *D* and Fay and Wu's *H* were significant at the 5% level. Using the partial sequences of *D. melanogaster* and *D. simulans*, the MK test was performed. Table 2 shows the 2×2 contingency table for the MK test. The MK test did not show any significant deviation from neutrality (*G*-value with Williams' correction = 2.589, $P = 0.10758$).

DISCUSSION

Using two strains with high and low sensitivity to trehalose, trehalose sensitivity was found to be a genetic dimorphism (TANIMURA *et al.* 1982). Our result indicates that the single-amino-acid change (Ala218Thr) in the

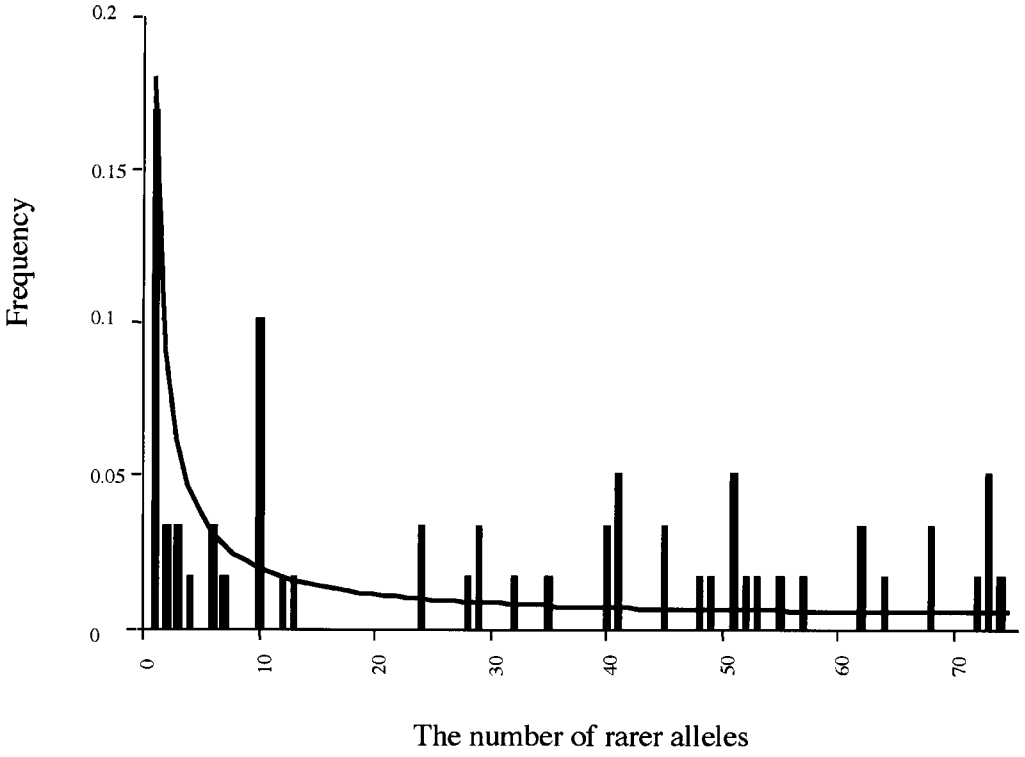


FIGURE 4.—Frequency spectrum of segregating nucleotides in the *Gr5a* gene. *x*- and *y*-axes indicate number of rarer variants and frequency, respectively. Solid curve and vertical bar indicate the expected and observed values, respectively.

Gr5a gene results in genetic dimorphism at the *Tre* locus. That is, genetic dimorphism can be explained in terms of the single SNP at position 926 in the *Gr5a* gene, which associates with trehalose sensitivity. We also found that both wild-type Tre^{+} (high sensitivity) and spontaneous mutant Tre^{01} (low sensitivity) alleles denoted by UENO *et al.* (2001) are naturally occurring variants and that their frequencies were 0.296 and 0.704, respectively, with respect to the causal SNP (G926A). The Tre^{+} and Tre^{01} alleles had alanine and threonine at amino acid position 218, respectively. Moreover, we found that there was significant genetic variation in trehalose sensitivity, and that it was continuously distributed. This observation indicates that trehalose sensitivity measured by the behavioral assay is a quantitative trait.

We found that apart from the *Gr5a* gene, other genetic factors are responsible for trehalose sensitivity measured by the two-choice preference test. The other genetic factors may involve additional gustatory receptor genes on the *X* chromosome and autosomes. Even if it is true, the effect of those gustatory receptors on trehalose sensitivity could not be large, because our results indicate that the single-amino-acid change of the gustatory receptor gene, *Gr5a*, has a major effect on trehalose sensitivity. Trehalose sensitivity measured by the two-choice preference test is a consequence of behavior of taste recognition. Therefore, apart from the gustatory receptors, many other genetic factors affecting processing of gustatory information must be included. Recent advances in quantitative trait loci mapping methods may enable us to identify these factors.

TABLE 2

2 × 2 contingency table for the MK test

	Fixed	Polymorphic
Synonymous	24	46
Replacement	7	29

G-value with Williams' correction = 2.589, $P = 0.10758$.

Considerable LD was observed in the *Gr5a* gene. The estimated minimum number of recombination events suggested the recombination rate is not low in this gene region. This is consistent with the estimate of the rate of crossing over in this region of the *X* chromosome (2.27 cM/Mb, ANDOLFATTO and WALL 2003). Therefore, LD was expected to decay rapidly. LD can be caused by admixture of subdivided populations, epistatic selection, or the maintenance of haplotypes by some form of balancing selection. In this study we found that only polymorphism at position 926 was associated with trehalose sensitivity, suggesting that there is no epistatic selection between polymorphism at position 926 and other polymorphisms in the *Gr5a* gene. We found significant interaction effect between sites 696 and 926 and between sites 926 and 1690. However, these sites were not in LD. Thus, epistatic selection could not be a main force to create the observed LD. Figure 5 shows the result of a sliding-window plot for silent changes along the sequence. A peak in the graph is expected to be centered around a

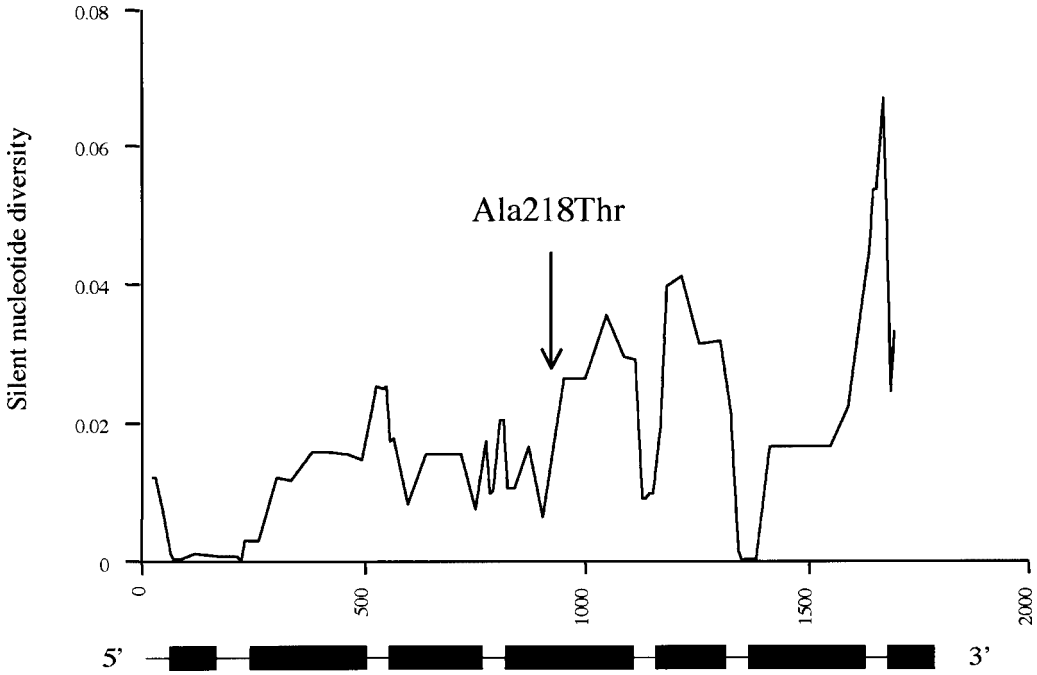


FIGURE 5.—Sliding-window plot of the nucleotide diversity at silent sites in *D. melanogaster*. The window size is 50 bp and step size is 25 bp. Solid boxes indicate exons and solid bars indicate the 5′-flanking region or introns. Arrow indicates the position with the Ala218Thr amino acid change.

site containing a balanced polymorphism (KREITMAN and HUDSON 1991). Figure 5 did not show such a peak being centered around site 926. Therefore, the Ala218Thr amino acid polymorphism is not likely to be a balanced polymorphism.

The power of tests of neutrality used in this study is low and their sensitivities are different (*e.g.*, SIMONSEN *et al.* 1995; AKASHI 1999). In particular, in the presence of recombination, the tests are too conservative (WALL 1999). Our sample showed evidence of recombination. To determine the critical values of the test statistics, 10,000 coalescent simulations with the observed level of recombination were performed. Tajima's *D* and Fay and Wu's *H* were positively and negatively significant, respectively. A significant Fay and Wu's *H*-value suggests recent hitchhiking events (MAYNARD SMITH and HAIGH 1974; KAPLAN *et al.* 1989). If it is true, Tajima's *D*-value is expected to be negative (BRAVERMAN *et al.* 1995; SIMONSEN *et al.* 1995). Thus, simple recent hitchhiking is not likely to explain the observed test statistic values. Combinations of recent hitchhiking and other evolutionary forces appear to be a better explanation. One of the alternatives is due to demographic factors. For instance, coalescent simulations using the ms program (HUDSON 2002) revealed that the observed test statistic values were possible under an island model with the observed level of recombination for a particular range of migration parameter (data not shown). With our present data, it is difficult to distinguish between the contributions of natural selection and demographic factors. To clarify this, it would be necessary to investigate the levels and patterns of variation at many other loci.

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LITERATURE CITED

AKASHI, H., 1999 Inferring the fitness effects of DNA mutations from polymorphism and divergence data: statistical power to detect directional selection under stationary and free recombination. Genetics **151:** 221–238.

ANDOLFATTO, P., and J. D. WALL, 2003 Linkage disequilibrium patterns across a recombination gradient in African *Drosophila melanogaster*. Genetics **165:** 1289–1305.

BRAVERMAN, J. M., R. R. HUDSON, N. L. KAPLAN, C. H. LANGLEY and W. STEPHAN, 1995 The hitchhiking effect on the site frequency spectrum of DNA polymorphisms. Genetics **140:** 783–796.

CHYB, S., A. DAHANUKAR, A. WICKENS and J. R. CARLSON, 2003 *Drosophila Gr5a* encodes a taste receptor tuned to trehalose. Proc. Natl. Acad. Sci. USA **100:** 14526–14530.

CLYNE, P. J., C. G. WARR and J. R. CARLSON, 2000 Candidate taste receptors in *Drosophila*. Science **287:** 1830–1834.

DAHANUKAR, A., K. FOSTER, W. M. VAN DER GOES VAN NATERS and J. R. CARLSON, 2001 A *Gr* receptor is required for response to the sugar trehalose in taste neurons of *Drosophila*. Nat. Neurosci. **4:** 1182–1186.

DUNIPACE, L., S. MEISTER, C. MCNEALY and H. AMREIN, 2001 Spatially restricted expression of candidate taste receptors in the *Drosophila* gustatory system. Curr. Biol. **11:** 822–835.

FU, Y. X., and W. H. LI, 1993 Statistical tests of neutrality of mutations. Genetics **133:** 693–709.

FAY, J. C., and C.-I WU, 2000 Hitchhiking under positive Darwinian selection. Genetics **155:** 1405–1413.

GILBERT, D. G., 1996 *SeqPup, Biosequence Editor and Analysis Platform* (http://iubio.bio.indiana.edu/).

HUDSON, R. R., 1987 Estimating the recombination parameter of a finite population model without selection. Genet. Res. **50:** 245–250.

Hudson, R. R., 2002 Generating samples under a Wright-Fisher neutral model of genetic variation. Bioinformatics **18:** 337–338.

Hudson, R. R., and N. L. Kaplan, 1985 Statistical properties of the number of recombination events in the history of a sample of DNA sequences. Genetics **111:** 147–164.

Kaplan, N. L., R. R. Hudson and C. H. Langley, 1989 The "hitchhiking effect" revisited. Genetics **123:** 887–899.

Kreitman, M., and R. R. Hudson, 1991 Inferring the evolutionary histories of the Adh and Adh-dup loci in *Drosophila melanogaster* from patterns of polymorphism and divergence. Genetics **127:** 565–582.

Maynard Smith, J., and J. Haigh, 1974 The hitch-hiking effect of a favorable gene. Genet. Res. **23:** 23–35.

McDonald, J. H., and M. Kreitman, 1991 Adaptive protein evolution at the *Adh* locus in *Drosophila*. Nature **351:** 652–654.

Nei, M., 1987 *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Robertson, H. M., C. G. Warr and J. R. Carlson, 2003 Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **100:** 14537–14542.

Rozas, J., J. C. Sachez-DelBarrio, X. Messegyer and R. Rozas, 2003 DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics **19:** 2496–2497.

Scott, K., R. Brady, Jr., A. Cravchik, P. Morozov, A. Rzhetsky *et al.*, 2001 A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. Cell **104:** 661–673.

Simonsen, K. L., G. A. Churchill and C. F. Aquadro, 1995 Properties of statistical tests of neutrality for DNA polymorphism data. Genetics **141:** 413–429.

Tajima, F., 1989 Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123:** 585–595.

Tanimura, T., K. Isono, T. Takamura and I. Shimada, 1982 Genetic dimorphism in the taste sensitivity to trehalose in *Drosophila melanogaster*. J. Comp. Physiol. **147:** 433–437.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin and D. G. Higgins, 1997 The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25:** 4876–4882.

Ueno, K., M. Ohta, H. Morita, Y. Mikuni, S. Nakajima *et al.*, 2001 Trehalose sensitivity in *Drosophila* correlates with mutations in and expression of the gustatory receptor gene *Gr5a*. Curr. Biol. **11:** 1451–1455.

Wall, J. D., 1999 Recombination and the power of statistical tests of neutrality. Genet. Res. **74:** 65–79.

Watterson, G. A., 1975 On the number of segregating sites in genetic models without recombination. Theor. Popul. Biol. **7:** 256–276.

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